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Lethal Concentration of Carbonate OF Ca as a Function of the Osmotic Potential of the Solution in Sunflower (*Heliantusannuus* L.)

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Abstract— *In order to know the effect of $CaCO_3$ in solution, sunflower seedlings cv. Victoria, an experiment was completely randomized, where five concentrations of calcium carbonate were evaluated to determine the lethal concentration (LC_{50}), pH and EC of the solution under laboratory conditions in the Universidad Tecnologica de Tehuacan, to simulate of excess Ca^{++} in the soils or nutrient solution. The results indicate, the LC_{50} was 62.8 mg $CaCO_3$ L^{-1}, so maximum values for pH, EC and calcium absorption, They were achieved at concentrations of 120 and 160 mg L^{-1} of $CaCO_3$. This work can be concluded, Sunflower can absorb the high levels of calcium and used as an alternative, for remediation of agricultural soils affected hard water and Ca^{++} salts.*

Keywords— osmotic potential, hard water, bioremediation.

I. INTRODUCTION

Osmotic potential is an important factor in the growth and development of crops, the absorption of water and nutrients depends largely on this,Because the fertilizers dissolved in the soil solution that will be absorbed by the plant,In large quantities cause high osmotic pressures, thus limiting their assimilation (Martínez*et al*., 2011; Parra *et al*., 2008).Soils that are irrigated with hard water contain amounts greater than 120 mg L^{-1} of Ca^{++} or Mg^{++},Increasing the osmotic potential of the soil, the absorption of water and nutrients(Sarabia*et al*. 2011),This significantly affects the agronomic and biological yield of crops susceptible to this factor such as: wheat, beans, soybean, strawberry and in severe cases, it causes death of the plant,Due to the null absorption of water, which can reach permanent wilt, by osmotic potential reaching values of -15.00 Bars (Argentel et al., 2008).Within the Asteraceae family, there are plants such as sunflower (*Helianthus annuus* L.) that have the potential to be considered as bioremediators of soils,By their ability to absorb heavy metals such as Pb^{++}, Cd^{++}, Ca^{++} and Mg^{++}, and their ability to grow in alkaline soils,Which have been affected by irrigation with hard water, such as Tehuacan, Puebla (Chico *et al*., 2012).For this reason in the present study the main objective was:To evaluate the effect of the osmotic potential of five solutions of $CaCO_3$ on sunflower seedlings (*Helianthus annuus* L.).The respective hypothesis was: $CaCO_3$ levels of 120 mg L^{-1} or higher, will affect the growth of sunflower seedlings (*Helianthus annuus* L.).

II. MATERIALS AND METHOD

2.1 Location of the experiment.

The present study was carried out under laboratory conditions, at the Universidad Tecnologica de Tehuacan, located in San Pablo TepetzingoTehuacan Puebla, Mexico at 18° 24'51" north latitude, 97 ° 20'00" longitude west and 1409 meters above sea level.

2.2 Genetic material.

The germplasm used were achenes of sunflower cv. Victoria free pollination,Which were donated by the germplasm bank of the Colegio de Postgraduados, Campus Montecillo, Which correspond to a sunflower subjected to stratified mass selection, intended for the production of oil.

2.3 Experimental design.

The design by which the response variables were evaluated was completely randomized and four replicates (5x4) = 20 experimental units,Valued under the mathematical model:

$Y_{ij} = \mu + T_i + \varepsilon_{ij}$ where:

Y_{ij}, is the response variable of the i-th osmotic potential of $CaCO_3$, in the j-th repetition.

μ, is the true overall mean.

T_i, is the effect of the i-th osmotic potential of $CaCO_3$ in the solution.

ε_{ij}, is the experimental error of the i-th osmotic $CaCO_3$ potential of the solution in the j-th repetition (Cochran and Cox, 2008).

2.4 Experimental unit and seedling management.

This consisted of a glass container of 325 ml capacity and a sunflower seedling.The seedlings were germinated in 200-well polystyrene trays,Using as substrate Peat Moss.When the seedlings had a height of 10 cm, they were transplanted into the glass vessel containing the osmotic $CaCO_3$ solution to start the experiment.

2.5 Preparation of the $CaCO_3$ solution and determination of the osmotic potential.

For the elaboration of the solution, de-ionized water was used, in order to reduce the bias in the data, by the presence of some ion.The calcium carbonate used was analytical grade.The concentrations used were prepared according to the following equation $\Psi_\pi = -CRT$, where:

Ψ_π,is the osmotic potential of the solution (atmospheres).

C,is the molarity of the solution (mol L^{-1}).

R, general gas constant0.08205 $\left(\frac{atm\ L}{mol\ ^oK}\right)$.

T, absolute temperature (°K).

The resulting solutions were:Control, 40, 80, 120 and 160 mg L^{-1} of $CaCO_3$.Whose osmotic potentials were:0; -0.030; -0.059; -0.088 and -0.12 atmospheres.Which were transformed to (0, -0.0030; -0.0050 y -0.0088 MPa) (Morales *et al*., 2010; Azcon and Talon, 2000; Salisbury y Ross, 2000).

2.6 *Response variables.*

- Lethal concentration (LC_{50}).

It was determined by counting in number of live seedlings, and transforming the values for each treatment to a percentage and with the help of a simple linear regression,50% was interpolated to obtain the LC_{50} and plot the corresponding curve (Zar, 2007; Steel and Torrie, 1990).

- pH and electrical conductivity of the solution.

The pH and electrical conductivity (dS m^{-1}) of the solution, was measured and recorded every 8 days over a period of 23 days with the aid of a Hanna potentiometer, model HI 991300.

- Calcium absorbed.

It was determined over a period of 23 days at 8 day intervals,With a Horiba model ionometer LAQUA B-721 for Ca^{++},Taking a leaf sample of the seedling that was representative of the middle phytomer and extracting the fluid from the petiole, to perform the corresponding measurement, and express the result in mg L^{-1} of Ca^{++}.

- SPAD units.

Determined with a Minolta-502 chlorophyll meter, taking the readings directly from the leaf blade (Loeza*et al*., 2016; Escalona*et al*., 2009).

When the response variables were significant,Tukey's mean comparison test was applied at a significance level of 5% of error probability with the help of the SAS program.

III. RESULTS AND DISCUSSION

3.1 Determination of the LC_{50}.

Figure 1 presents the percentage of survival of the sunflower seedlings, which were subjected to different concentrations of calcium carbonate.In it can be seen that the mathematical model of adjustment was linear decreasing,as indicated by the slope of the curve -0.49 thus indicating that for each (mg L^{-1}) of $CaCO_3$ applied to the solution, the percentage of survival of sunflower seedlings, decreases by 0.49%.Interpolation in model P = -0.49C + 80.8 indicated that the LC_{50} for sunflower was 62.8 mg L^{-1} of calcium carbonate.Data that were supported by the good fit of the model, since the coefficient of determination was high 0.99 and highly significant.According to the model obtained the sunflower cv. Victoria, can withstand up to 140 mg L^{-1} of $CaCO_3$,Presenting survival values of 12.2%, while values of 160 mg L^{-1} or higher,Cause the death of the

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sunflower seedlings,Suggesting that this species is susceptible to this dose of $CaCO_3$.

This response was studied by Arbelo*et al.* (2006), who mention that the alkalinity of soils caused by Na^+ or Ca^{++} in arid zones,limit the fertility of this resource due to the alteration of its physical properties such as:Structure, in addition to crusting and surface sealing, in addition to the low water filtration capacity, facilitating the processes of desertification in these areas,Becoming one of the main causes of the abandonment of arable land.

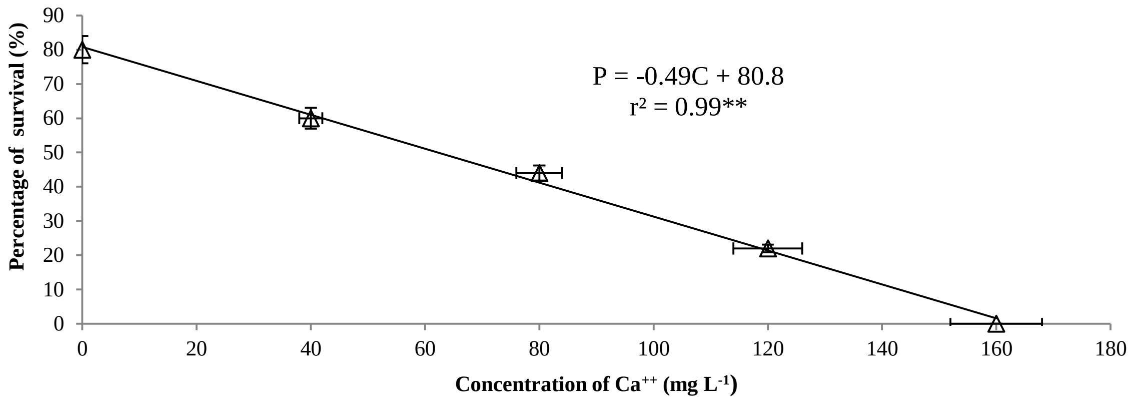

*Fig.1:Percentage survival curve in sunflower seedlings (Helianthus annuus L.), under five levels of $CaCO_3$.Technological University of Tehuacán.Spring, 2016.P, percentage of survival; C, concentration; **; *; n.s, significant at 0.01; 0.05 and not significant.*

3.2 pH and conductivity.

The pH behavior of the solution as a function of time is presented in figure 2,it can be seen that the high levels of $CaCO_3$ 160 and 140 mg L^{-1} were adjusted to quadratic models.At the 160 mg L^{-1} concentration 8 to 16 days after transplantation, the pH behaved in an increasing manner, in order to present the inflection point where the concavity of the curve was negative until 23 days after of transplantation (dat). Thus decreasing from 10.5 to 10.0 for 160 mg L^{-1}, while in 120 mg L^{-1}, the pH increased from 9.7 to 9.9. Thus under this trend, the concentrations 80, 40 and control were adjusted to a growing linear model until the end of the experiment.

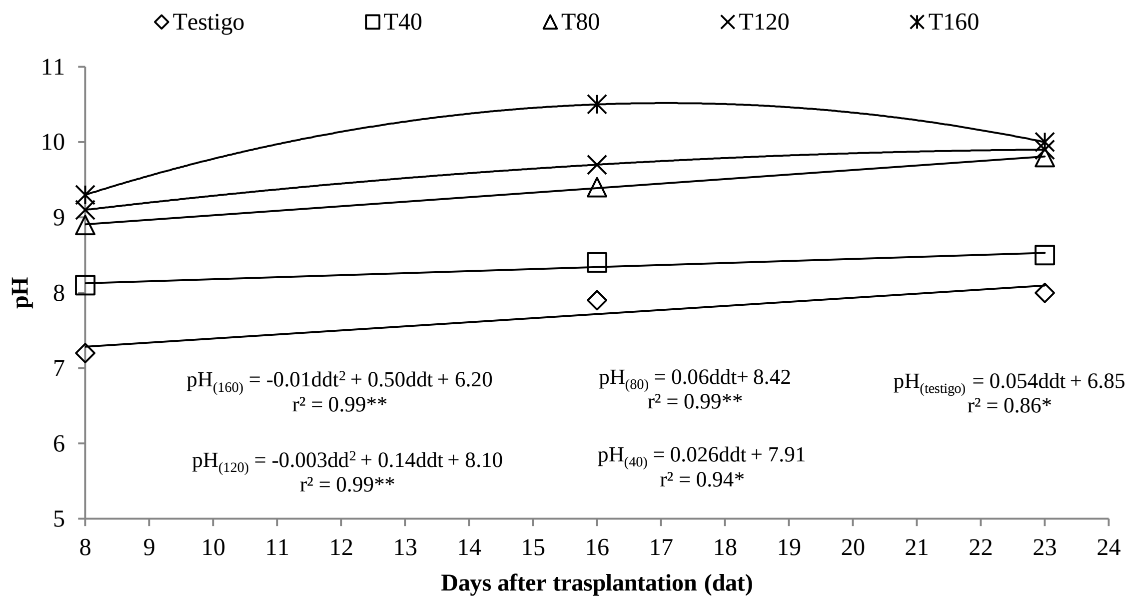

*Fig.2: pH dynamics in five solutions of $CaCO_3$, in sunflower (Helianthus annuus L.) cv. Victory. Universidad Tecnologica de Tehuacan. Spring 2016.**; *; n.s, significant at 0.01; 0.05 and not significant.*

Regarding the electrical conductivity, the maximum values were presented in the high doses 120 and 160 mg L^{-1} of $CaCO_3$ for the three dates, resulting in addition, statistically equal.Thus the behavior of this for the doses in question was increasing, as shown in Table 1.On the contrary the low doses occurred in the control at 16 and 23 days after of plantation, with 3.45 and 3.77 dS m^{-1}.

This phenomenon is due, to that the evapotranspiration of the water concentrates the quantity of salts like the $CaCO_3$ that are dissolved in the water or nutritive solution,Thus increasing the electrical conductivity, and this can reach values of 7 or 8 dS m^{-1}, when this variable is not controlled (Carrasco *et al*., 2007; Lara, 2000).

Table.1: Analysis of variance and multiple comparison test for electrical conductivity of five concentrations of $CaCO_3$ in sunflower (Helianthus annuus L.) cv. Victory. Universidad Tecnologica de Tehuacan. Spring 2016.

Treatment	dat		
	8	16	23
mg L^{-1}		dS m^{-1}	
control	2.80 c	3.45 d	3.77 d¶
T_{40}	4.00 b	4.20 c	4.67 c
T_{80}	5.20 a	5.45 b	5.85 b
T_{120}	5.30 a	5.70 ab	6.25 ab
T_{160}	5.40 a	6.05 a	6.60 a
HSD	0.54**	0.35**	0.54**
CV%	5.4	3.24	4.59

¶Average within columns with the same literal are statistically the same according to Tukey at P≤0.05; dat, days after transplantation; HSD, honestly significant difference; CV, coefficient of variation; **; *; n.s, significant at 0.05; 0.01 and not significant.

3.3 Calcium absorbed.

The analysis of variance for absorbed calcium,Shows that there were highly significant differences between treatments at 16 and 23 dat (Table 2).At this time the treatment where 160 mg L^{-1}, was the one who absorbed the largest amount of Ca^{++} as shown by the petiole extract, absorbing 59.92 and 61.35 mg L^{-1} of Ca^{++}.Regarding the lower absorption of calcium,This occurred in the control treatment,Who only absorbed 40.75 and 41.10 mg L^{-1} at the mentioned dates.This response has been studied by Fabela*et al.* (2006),Who mentions that the excess of calcium in soils with high presence of this nutriment, is absorbed by the plants that grow in this,Inhibiting the assimilation of micronutrients like Fe^{++} and Mg^{++},Manifesting itself in a chlorosis that is very marked in the leaves,Also mention that calcium can be measured and monitored with ionometers that allow us to know their levels in the crop.The above is of great importance,Due to deficiencies of this nutrient and to affect the quality, in the fruits as in tomato (Bugarín*et al*., 2002).

Table.2: Analysis of variance and multiple comparison test for calcium absorption of five concentrations of $CaCO_3$ in sunflower (Helianthus annuus L.) cv. Victory. Universidad Tecnologica de Tehuacan. Spring 2016.

Treatment	dat		
	8	16	23
mg L^{-1}		mg L^{-1}	
control	32.57 a	40.75 e	41.10 e¶
T_{40}	33.35 a	45.42 d	45.05 d
T_{80}	33.65 a	50.25 c	50.55 c
T_{120}	33.42 a	55.25 b	56.02 b
T_{160}	33.42 a	59.92 a	61.35 a
HSD	2.63 n.s	2.95**	2.37**
CV%	3.62	2.68	2.13

¶Averages within columns with the same literal are statistically the same according to Tukey at P≤0.05; dat,

days after transplantation; HSD, honestly significant difference; CV, coefficient of variation; **; *; n.s, significant at 0.05; 0.01 and not significant.

IV. CONCLUSIONS

Derived from the results of the present investigation, the following conclusions were reached.

- The highest values of pH in the solution were presented in the high concentrations of $CaCO_3$ and 120 mg L^{-1}.
- High pH levels occurred at 16 and 23 dat, and were a consequence of evapotranspiration.
- High levels of electrical conductivity, were also the result of high levels of $CaCO_3$ and evapotranspiration.
- The highest absorption of Ca^{++}, was in the concentrations of 160 and 120 mg L^{-1}.
- Sunflower is a plant that can withstand high levels of $CaCO_3$, extracting this from the soil solution.
- Sunflower may be an alternative to extract calcium from the soil in soils that have been affected by excess of this nutrient.

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